ISSN: 0975-8232

IJPSR (2010), Vol. 1, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received 12 March, 2010; received in revised form 28 May, 2010; accepted 11 June, 2010

IN VITRO PROPHYLACTIC CATARACT PREVENTION STUDY ON GLUCOSE INDUCED CATARACT BY QUERCETIN AND ALPHA- TOCOPHEROL

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Keywords:

Quercetin,

Alpha- tocopherol,

anticataract

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ABSTRACT

The antioxidants such as quercetin and alpha-tocopherol were subjected for *in vitro* prophylactic cataract prevention study on glucose induced cataract model. Goat lenses were incubated in artificial aqueous humor containing 55 mM glucose with test drugs in different concentrations at room temperature for 72 h. Biochemical parameters studied in the lens were electrolytes (Na^+, K^+), Na^+- K^+- ATPase activity, malondialdehyde (MDA) and proteins. Glucose induced opacification of goat lens began 8-10 hrs after incubation and was complete in 72-80 hrs. Cataractous lenses showed higher Na^+, MDA ($P<0.001$), lower Na^+- K^+- ATPase activity, and water-soluble protein content. Lenses treated with test drugs has shown higher protein content and prevented formation and progress of cataract by glucose, as evidenced by biochemical parameters.

INTRODUCTION: Cataract is the opacification of the lens, develops later in the life and it is most likely the consequence of decades of accumulated damage to long lived lens protein [1, 2]. It is a multifactorial disease occurs mainly due to formation of large protein aggregates in the lens. The research has shown that post translational modification of lens crystallins such as oxidation, glycation, carbamylation, transamidation, phosphorylation and proteolysis lead to clouding of lens [2, 3]. Oxidative mechanism plays an important role in biological phenomena including cataract formation.

The formation of superoxide radicals in the aqueous humor and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to formation of cataract [3]. The enzyme aldose reductase also plays an important role in pathogenesis of cataract. The aldose reductase acts on the sugars like glucose, galactose and xylose and convert them into their respective alcohols. These alcohols, also known as polyols accumulate within the lens there by producing osmotic effects. Since polyols are not capable of diffusing out easily nor metabolizes rapidly and causes hyper tonicity responsible for formation of cataract[4].

The higher glucose level also leads the formation of superoxide radical and H_2O_2 [5]. The research is also implicated that Na^+- K^+- ATPase activity is important in maintaining ionic equilibrium in the lens, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis [6]. A number of drugs were tried for anticataract activity such as aldose reductase inhibitors, NSAIDS, calpain inhibitors, antiglycatics, anti-oxidants of natural and synthetic origin and a group of miscellaneous agents but none are found effective. The drugs such as quercetin and alpha- tocopherol have been found to afford protection from free radical damage in many experimental conditions [7]. Therefore, this study was undertaken to find the efficacy of quercetin and alpha- tocopherol in the prevention of experimental cataract induced by glucose.

MATERIALS AND METHODS:

Chemicals: Quercetin and Alpha- tocopherol was obtained as gift sample from Novepha Company Limited, Shangai- China. Other chemicals used in present study were analytical grade.

Eye Balls: Goat eye balls were used in the present study. They were obtained from the slaughterhouse immediately after slaughter and transported to laboratory at 0-4 degree Celsius. The study was approved by Institutional Animal Ethics Committee.

Preparation of Lens Culture: The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl - 140 mM, KCl - 5 mM, $MgCl_2$ - 2 mM, $NaHCO_3$ - 0.5 mM, $NaH(PO_4)_2$ - 0.5 mM, $CaCl_2$ - 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55 mM was used to induce cataract [8].

Test Drug Concentration and Groups: Three concentrations of the test drug were chosen. Concentrations were in such a way that the

middle dose was IC50 concentration of the test drug, high dose which was twice that of middle, and the last was 50% of middle concentration.

A total of 80 lenses were divided into following categories (n=10 in each category):

Group I: Normal lens [Control (Glucose 5.5 mM)]

Group II: Glucose 55 mM

Group III:
A: Glucose 55 mM + Quercetin1.2 mcg/ml
B: Glucose 55 mM + Quercetin 2.4 mcg/ml
C: Glucose 55 mM + Quercetin 4.8 mcg/ml

Group IV:
A: Glucose 55 mM + Alpha-tocopherol 3.6 mcg/ml
B: Glucose 55 mM + Alpha-tocopherol 7.3 mcg/ml
C: Glucose 55 mM + Alpha-tocopherol 14.6 mcg/ml

Homogenate preparation: After 72 h of incubation, homogenate of lenses was prepared in Tris buffer (0.23M, pH 7.8) containing $0.25X10^{-3}$sub M EDTA and homogenate adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4).

Biochemical estimation: Electrolyte (Na^+ & K^+) estimation was done by flame photometry. Na^+-K^+-ATPase activity was assessed by the method of *Unakar & Tsui* [9] and protein by *Lowry's method* [10]. The degree of oxidative stress was assessed by measuring the MDA levels by *Wilbur's method* [11].

Visual Evaluation: Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a lens as a measure of lens opacity.

Statistical analysis: The data was presented as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by post hoc- Dunnett's test using Graph Pad Prism software, version 4.01

RESULTS AND DISCUSSION: Incubation of lenses with glucose 55 Mm has shown opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the center, with complete opacification at the end of 72 hrs. The visual evaluation has shown that the test drugs retard the progression of lens opacification, compared with Group I and II.

Group II has shown significantly higher Na^+ ($P<0.05$), lower K^+ ($P<0.001$) and lower Na^+-K^+-ATPase activity ($P<0.001$) compared with Group 1 (table 1). Both Group III and IV, has shown significantly high K^+ ($P<0.001$), while Na^+-K^+-ATPase activity was significantly higher ($P<0.001$) with concentrations of middle and last, compared with Group II (table 1). Both test drug treated groups showed a lower Na^+ compared with Group II at all three concentrations, but was not statistically significant. Group II has also shown significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate ($P<0.001$) and very high MDA ($P<0.001$) compared with Group I (table 2).

TABLE 1: Na^+, K^+ and Na^+- K^+ -ATPase ACTIVITY IN LENS HOMOGENATE AFTER 72 h OF INCUBATION

STUDY GROUPS	Na^+ [meq/gm]	K^+ [meq/gm]	Na^+ -K^+-ATPase ACTIVITY [µgP/gm]
Group I	52.9 ± 56.1*	10.5 ± 1.5***	41.8 ± 2.2***
Group II	209.7 ± 29.7	6.5 ± 0.3	17.7 ± 4.9
Group III			
A	182.5 ± 28.9	9.5 ± 2.2**	23.8 ± 9.8
B	187.5 ± 29.4	9.4 ± 1.8*	34.5 ± 7.0***
C	180.0 ± 40.4	10.6 ± 2.3***	34.4 ± 9.5***
Group IV			
A	177.5 ± 38.0	9.5 ± 2.5**	24.7 ± 10.7
B	183.0 ± 41.4	9.6 ± 2.7**	37.3 ± 5.5***
C	182.0 ± 42.7	9.2 ± 2.0*	32.9 ± 10.5***

Values are mean ± SD. n=10 for each group. *P<0.05, **P<0.01 and ***P<0.001 as compared with their corresponding value in glucose 55 mM group

TABLE 2: PROTEINS (TOTAL PROTEINS AND WATER SOLUBLE PROTEINS) AND MALONDIALDEHYDE (MDA) IN LENS HOMOGENATE AFTER 72 H OF INCUBATION

STUDY GROUPS	TOTAL PROTEINS [mg/gm]	WATER SOLUBLE PROTEINS [mg/gm]	MDA [nmole/gm]
Group I	224.8 ± 32.9***	94.1 ± 18.4**	2.9 ± 1.1***
Group II	162.8 ± 29.1	61.6 ± 29.4	60.7 ± 20.0
Group III			
A	165.7 ± 31.0	69.0 ± 23.8	42.8 ± 12.2**
B	203.8 ± 29.0**	71.3 ± 21.7	32.5 ± 8.7***
C	202.4 ± 17.1*	70.7 ± 25.4	24.3 ± 13.1***
Group IV			
A	185.4 ± 45.6	64.7 ± 28.0	38.37 ± 14.3***
B	214.9 ± 27.7***	65.7 ± 25.8	27.4 ± 7.4***
C	210.8 ± 23.5**	69.9 ± 29.3	26.4 ± 9.1***

Values are mean ± SD. n=10 for each group. *P<0.05, **P<0.01 and ***P<0.001 as compared with their corresponding value in glucose 55 mM group

Group III and IV had significantly higher concentrations of total lens proteins at middle and last concentrations, compared with Group II. At the same time, they had higher water-soluble proteins at all three concentrations of test drugs, compared with Group II. However, the difference was not significant statistically. [Table 2] MDA levels were found to be very high in Group II, compared with Group I. Group I and II had significantly reduced MDA content ($P<0.01$) at all the three concentrations, compared with Group II (table 2).

The study has shown that higher Na^+ - K^+- ATPase activity, total and water-soluble proteins and K^+ ions whereas lower concentrations of Na^+ ions with test drug treated groups. Therefore, the test drugs seems to prevent the alteration of Na^+ and K^+ imbalance, which may be due to a direct effect on lens membrane Na^+-K^+-ATPase [12, 13] or indirect effect through their free radical scavenging activity. In this study MDA; an indicative of oxidative stress [14], levels were significantly higher in high glucose (55 mM) group, compared with normal control group.

The MDA levels were significantly less in the test drug treated groups at all concentrations. The test drugs have also been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration. Incubation in presence of high glucose (55 mM) concentration simulates a state of clinical diabetes. A preventive role of test drugs suggest in the preventing and/or retarding the progression of diabetic cataracts. However, higher concentrations of quercetin and alpha- tocopherol may show better anticataract activity, and further evaluation with higher concentrations is required. This *in vitro* study may not directly correlate with the *in vivo* conditions. Therefore, *in vivo* studies in different animal models are required for further elucidation.

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